Unsupervised Lab

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Data Description

gdat is Gene Expression Data, n = 445 patients x p = 353 genes

- Only 353 genes with somatic mutations from COSMIC are retained ## Data is Level III TCGA BRCA RNA-Sequencing gene expression data that have already been pre-processed according to the following steps:
- Reads normalized by RPKM
- Corrected for overdispersion by a log-transformation (1 + data)
- Short gene name labels are given as the column names

cdat is Clinical Data, n = 445 patients x q = 6 clinical features

- Subtype - denotes 5 PAM50 subtypes including Basal-like, Luminal A, Luminal B, HER2-enriched, and Normal-like
- ER-Status - estrogen-receptor status
- PR-Status - progesterone-receptor status
- HER2-Status - human epidermal growth factor receptor 2 status
- Node - number of lymph nodes involved
- Metastasis - indicator for whether the cancer has metastasized

Problems

Problem 1 - Dimension reduction

1a - Apply PCA, NMF, ICA and MDS, UMAP, and tSNE to this dataset. Compare and contrast the results using these methods.
1b - Relate the dimension reduction results with the clinical data. Is any clinical information reflected in the lower dimensional spaces?

1c - Overall, which dimension reduction method do you recommend for this data set and why?

Problem 2 - Clustering

2a - Apply various clustering algorithms such as K-means (explore different K), hierarchical clustering (explore different linkages), NMF, and biclustering. Compare the clustering results using these methods.

2b - Relate the clustering results with the clinical data. Can the clustering algorithm recover some of the clinical information such as cancer subtypes?

2c - Use Consensus Clustering to help Validate Clustering Results

2c - Overall, which clustering method(s) do you recommend for this data set and why?

Problem 3 - Multiple comparisons

3a - Identify important genes to differetiate ER postive and negative, PR postive and negative, HER2 postive and negative, metastasis status.

3b - Try different procedures to adjust for multiple comparisons.

3c - Examine the lists of genes identified using different methods for each clinical response. Which method is best? Why?

Problem 5 - Graphical models

5a - Use graphical models to explore interactions among genes. Are any of the well-connected genes related to patterns previously identified?

Problem 6 - Visulaization

6a - Visualize this data using multiple approaches.

6b - Prepare the "best" visual summary of this data.

Problem 7 - Exploratory Data Analysis Summary

7a - What is the most interesting finding?

7b - Is this finding consistent and stable?

7c - Prepare a visual summary that best illustrates this interesting finding.

R scripts to help out with the BRCA case study Lab Don't peek at this if you want to practice coding on your own!!

Load Data

```
load("UnsupL_SISBID_2020.Rdata")
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.6.2
```

```
library(ConsensusClusterPlus)
library(kknn)
library(GGally)
```

```
## Warning: package 'GGally' was built under R version 3.6.2
```

```
## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg   ggplot2
```

```
library(umap)
```

```
## Warning: package 'umap' was built under R version 3.6.2
```

```
library(Rtsne)
library(igraph)
```

```
##
## Attaching package: 'igraph'
```

```
## The following objects are masked from 'package:stats':
##
##     decompose, spectrum
## The following object is masked from 'package:base':
##
##     union
```

```
library(XMRF)
library(spacejam)
```

```
## Loading required package: splines
```

```
## Loading required package: Matrix
```

Explore Data

```
dim(gdat)
```

```
## [1] 445 353
```

```
dim(cdat)
```

```
## [1] 445   6
```

```
table(cdat$Subtype)
```

```
##
##    Basal-like HER2-enriched     Luminal A     Luminal B   Normal-like
##            79            53           200           106             7
```

```
table(cdat$ER)
```

```
##
##               Indeterminate                    Negative
##                           2                         100
##               Not Performed Performed but Not Available
##                           2                           2
##                    Positive
##                         339
```

```
table(cdat$PR)
```

```
##
##               Indeterminate                    Negative
##                           3                         147
##               Not Performed Performed but Not Available
##                           2                           2
##                    Positive
##                         291
```

```
table(cdat$HER2)
```

```
##
##     Equivocal      Negative Not Available      Positive
##             5           370             4            65
```

```
table(cdat$Node)
```

```
##
##   0   1   2   3
## 221 146  54  23
```

```
table(cdat$Metastasis)
```

```
##
```

```
##   0   1
## 427  11
```

```
table(cdat$ER,cdat$PR)
```

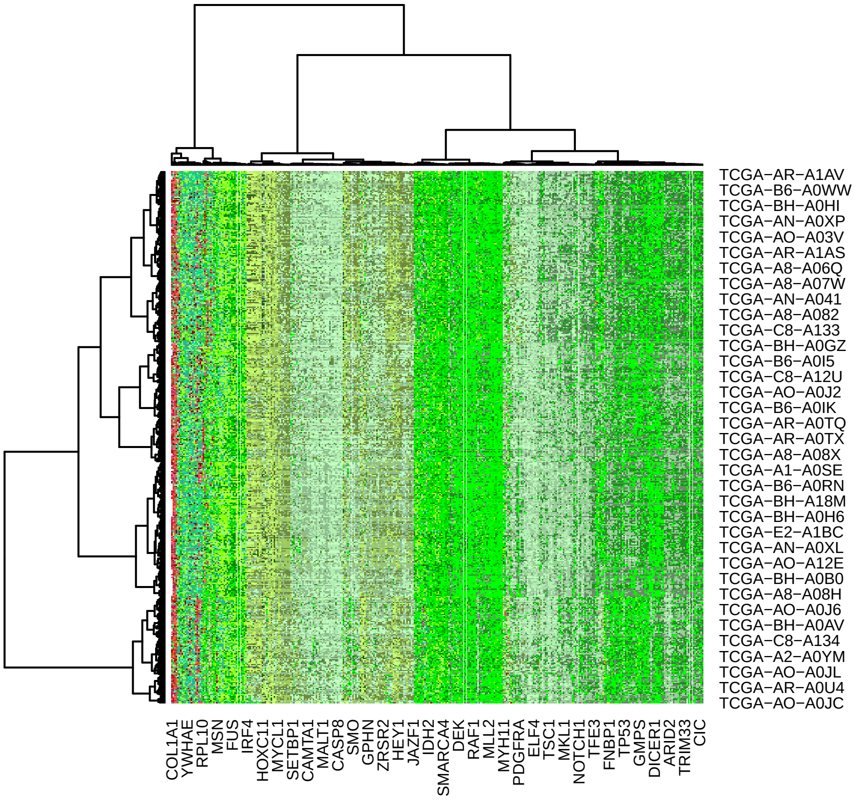
```
##
##                              Indeterminate Negative Not Performed
##   Indeterminate                          0        1             0
##   Negative                               1       93             0
##   Not Performed                          0        0             2
##   Performed but Not Available            0        0             0
##   Positive                               2       53             0
##
##                              Performed but Not Available Positive
##   Indeterminate                                        0        1
##   Negative                                             0        6
##   Not Performed                                        0        0
##   Performed but Not Available                          2        0
##   Positive                                             0      284
```

#cluster heatmap - biclustering

```
#cluster heatmap - biclustering
aa = grep("grey",colors())
bb = grep("green",colors())
cc = grep("red",colors())
gcol2 = colors()[c(aa[1:2],bb[1:25],cc[1:50])]
```

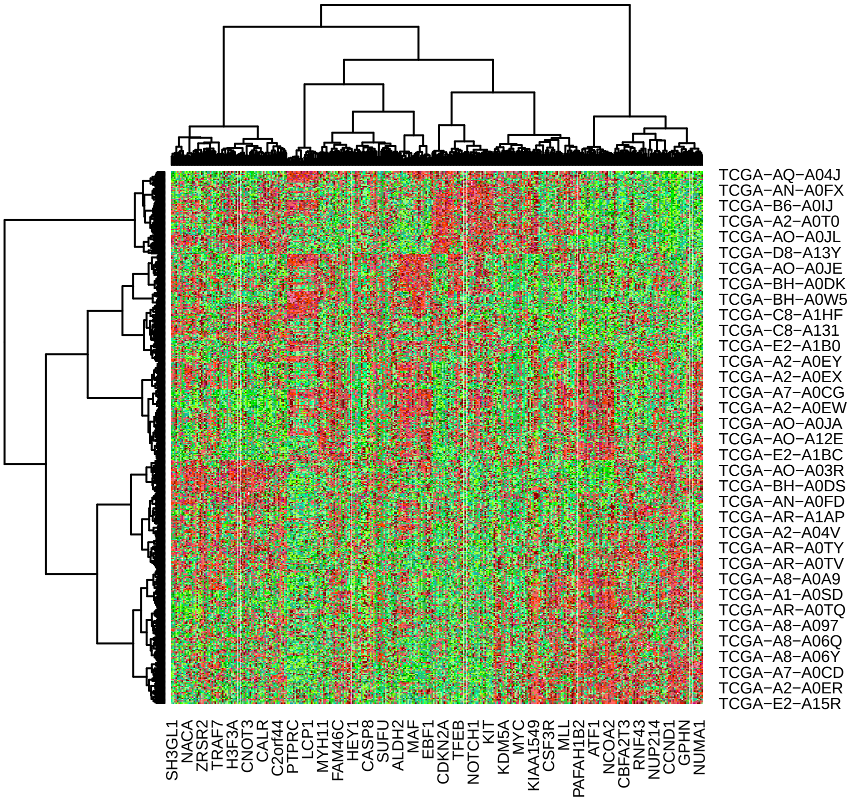
Without scaling

```
heatmap(gdat,col=gcol2,hclustfun=function(x)hclust(x,method="ward.D"))
```



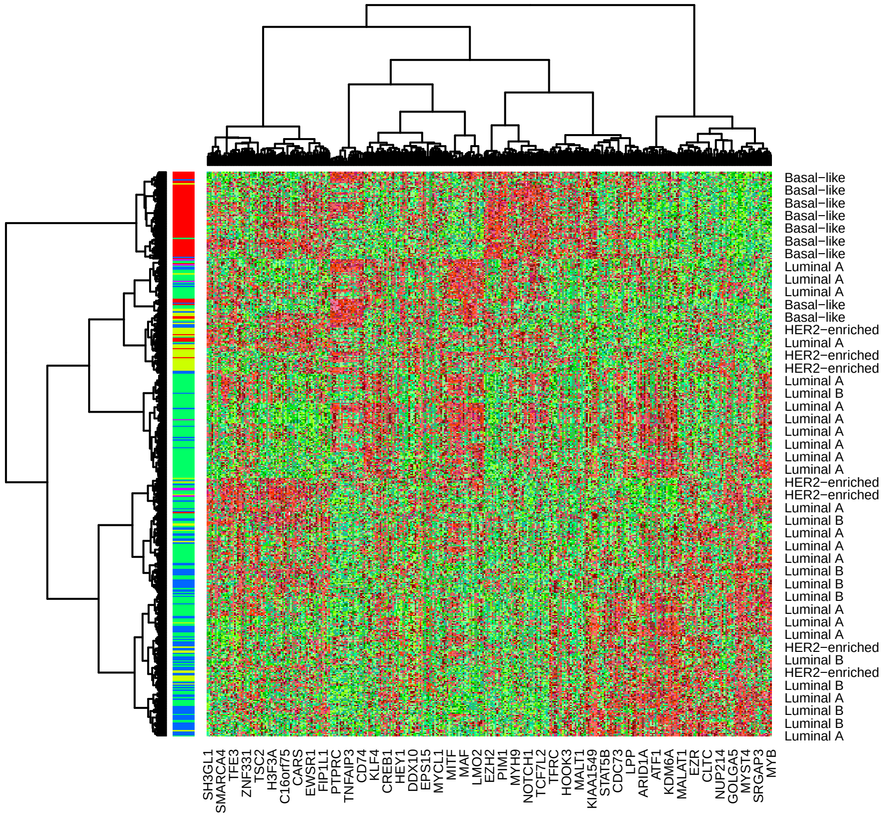
With scaling

```
heatmap(scale(gdat),col=gcol2,hclustfun=function(x)hclust(x,method="ward.D"))
```



```
Cols=function(vec){cols=rainbow(length(unique(vec)))
                    return(cols[as.numeric(as.factor(vec))])}

heatmap(scale(gdat),col=gcol2,hclustfun=function(x)hclust(x,method="ward.D"),labRow=cdat$Subtype,RowSid
```



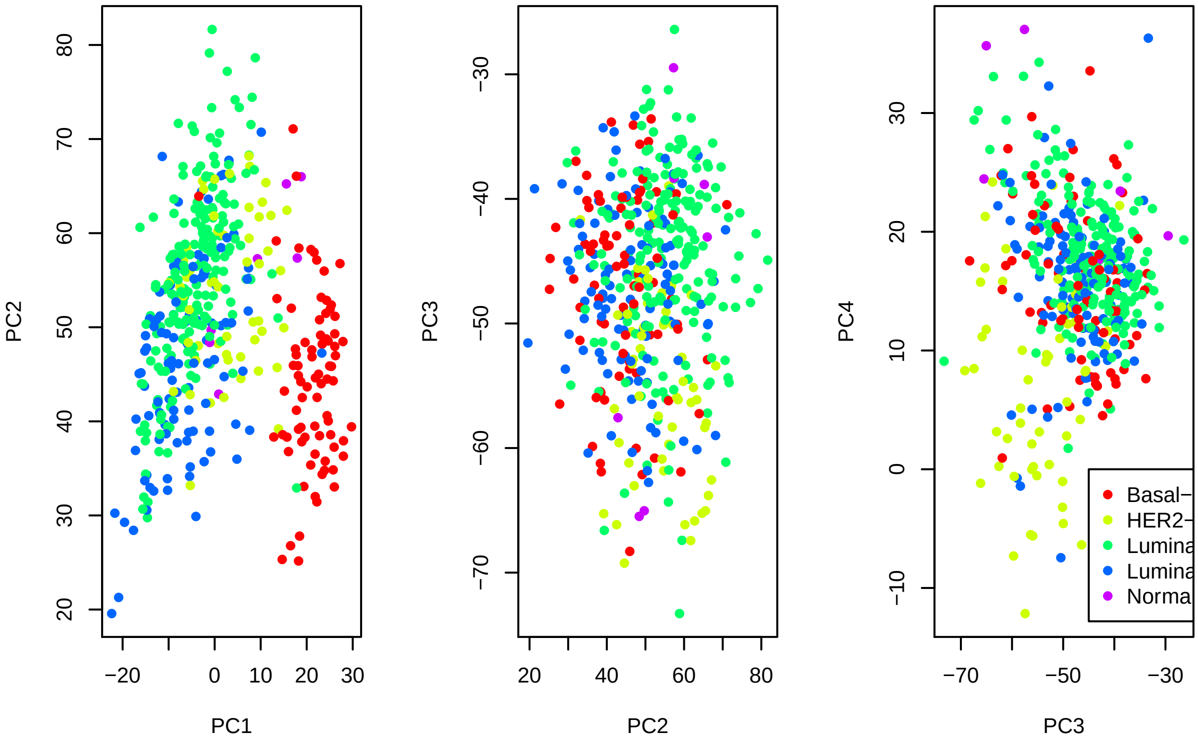
#Dimension Reduction

PCA

```
Cols=function(vec){cols=rainbow(length(unique(vec)))
                   return(cols[as.numeric(as.factor(vec))])}

sv = svd(scale(gdat,center=TRUE,scale=FALSE))
V = sv$v
Z = gdat%*%V

K = 3
pclabs = c("PC1","PC2","PC3","PC4")
par(mfrow=c(1,K))
for(i in 1:K){
  j = i+1
  plot(Z[,i],Z[,j],pch=16,xlab=pclabs[i],ylab=pclabs[j],col=Cols(cdat$Subtype))
}
legend(-45,0,pch=16,col=rainbow(5),levels(cdat$Subtype))
```



Pairs Plot

```
PC1<-as.matrix(Z[,1])
PC2<-as.matrix(Z[,2])
PC3<-as.matrix(Z[,3])
PC4<-as.matrix(Z[,4])
PC5<-as.matrix(Z[,5])

pc.df.cdat<-data.frame(Subtype = cdat$Subtype, PC1, PC2, PC3, PC4, PC5)
ggpairs(pc.df.cdat, mapping = aes(color = Subtype))
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
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## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```
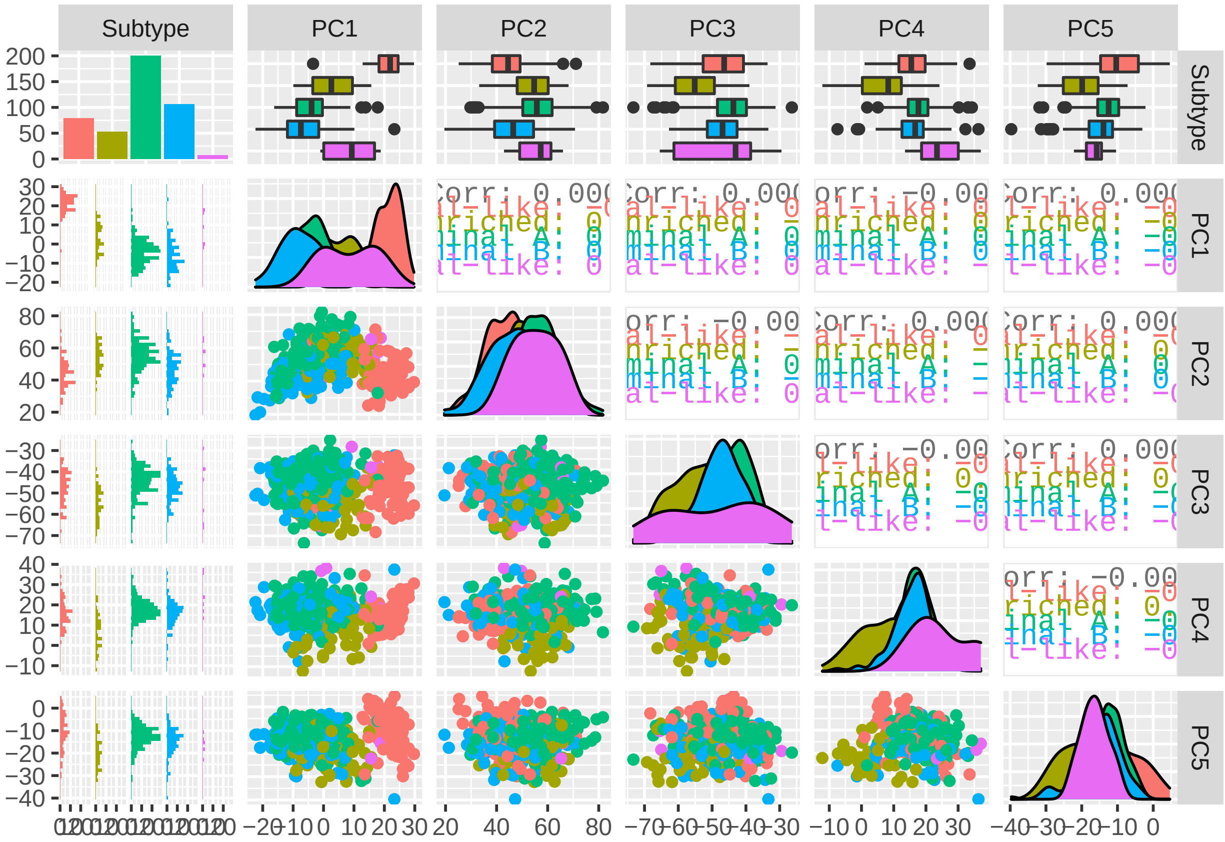
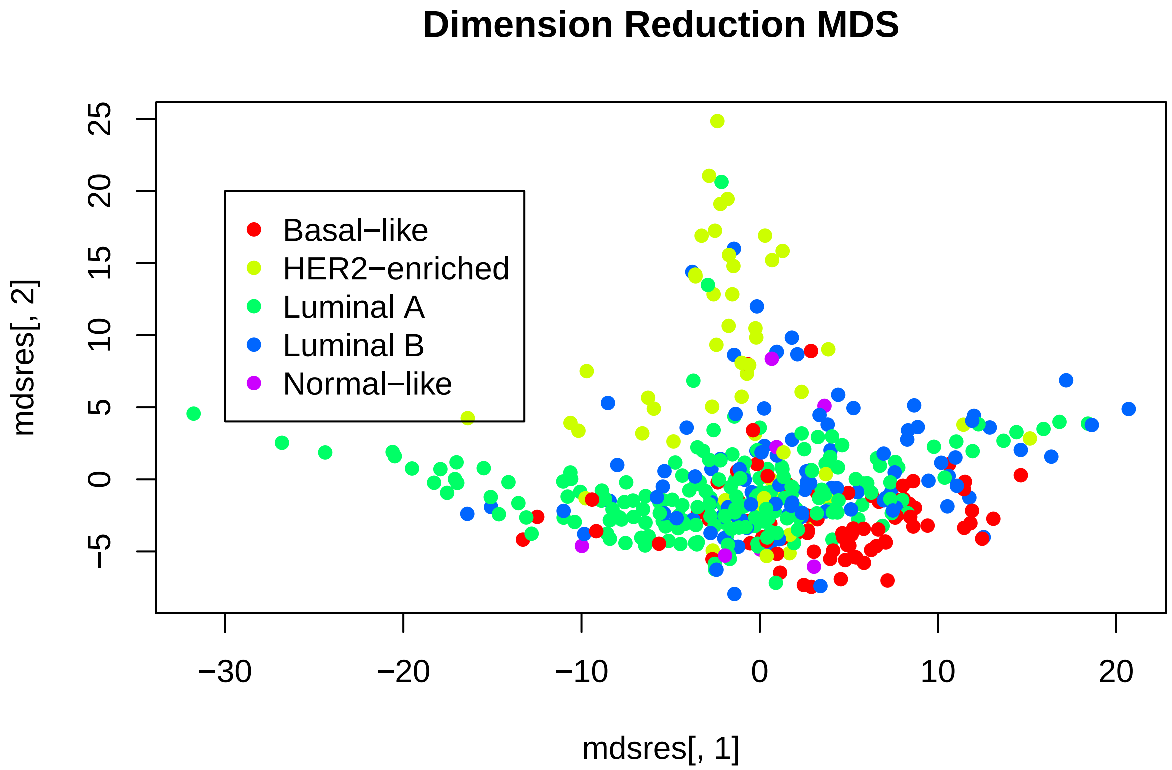



MDS

```
Dmat = dist(gdat,method="maximum")
mdsres = cmdscale(Dmat,k=2)
plot(mdsres[,1],mdsres[,2],pch=16,col=Cols(cdat$Subtype), main = "Dimension Reduction MDS")
legend(-30,20,pch=16,col=rainbow(5),levels(cdat$Subtype))
```

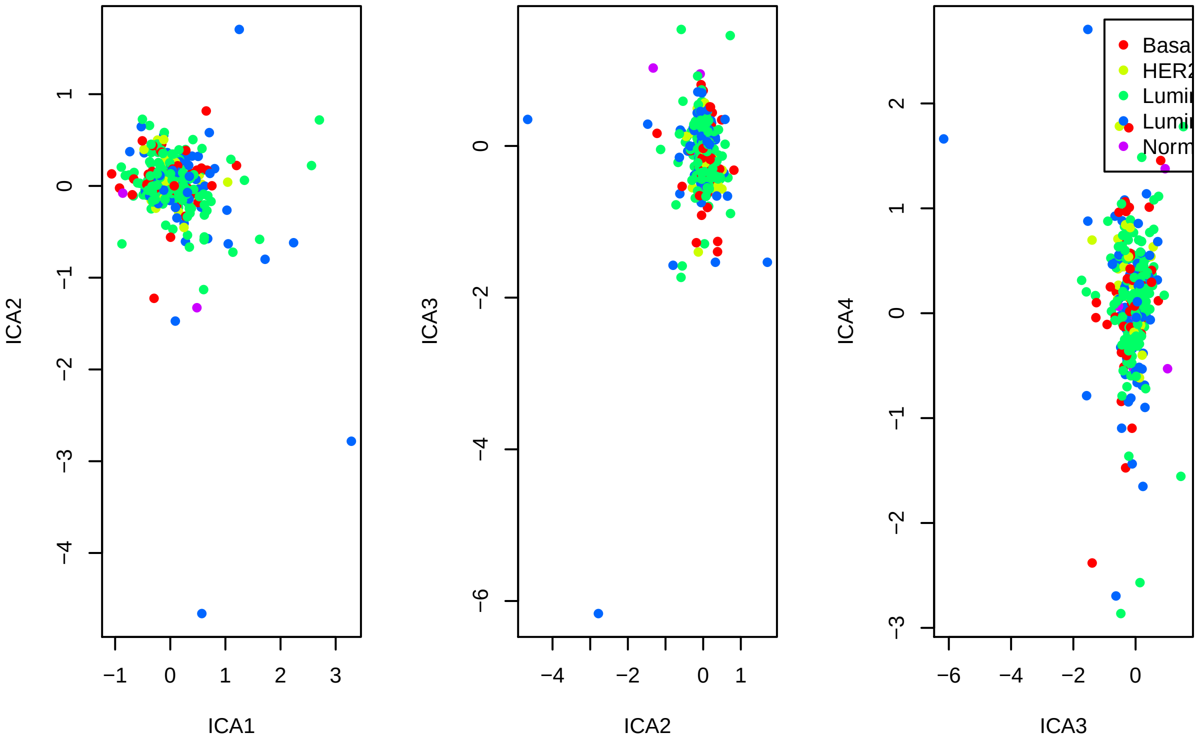


ICA

```r
require("fastICA")
```

```
## Loading required package: fastICA
```

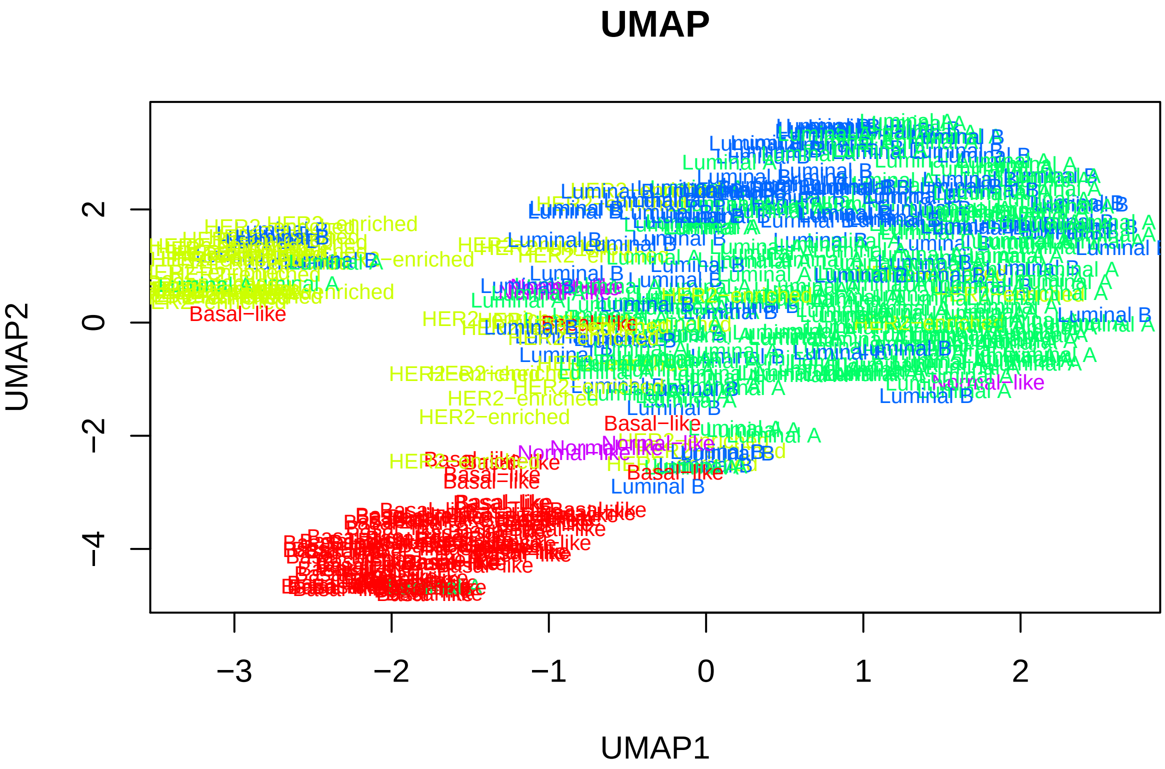
```r
K = 4
icafit = fastICA(gdat,n.comp=K)

kk = 3
pclabs = c("ICA1","ICA2","ICA3","ICA4")
par(mfrow=c(1,kk))
for(i in 1:kk){
  j = i+1
  plot(icafit$A[i,],icafit$A[j,],pch=16,xlab=pclabs[i],ylab=pclabs[j],col=Cols(cdat$Subtype))
}
legend(-1,2.8,pch=16,col=rainbow(5),levels(cdat$Subtype))
```



UMAP

```
gdat.umap = umap(gdat)
plot(gdat.umap$layout[,1], y =gdat.umap$layout[,2], type = "n", main = "UMAP", xlab = "UMAP1", ylab = "U
text(gdat.umap$layout[,1], y =gdat.umap$layout[,2], type = "n", cdat$Subtype, col=Cols(cdat$Subtype), c
```

```
## Warning in text.default(gdat.umap$layout[, 1], y = gdat.umap$layout[, 2], :
## graphical parameter "type" is obsolete
```



#Clustering

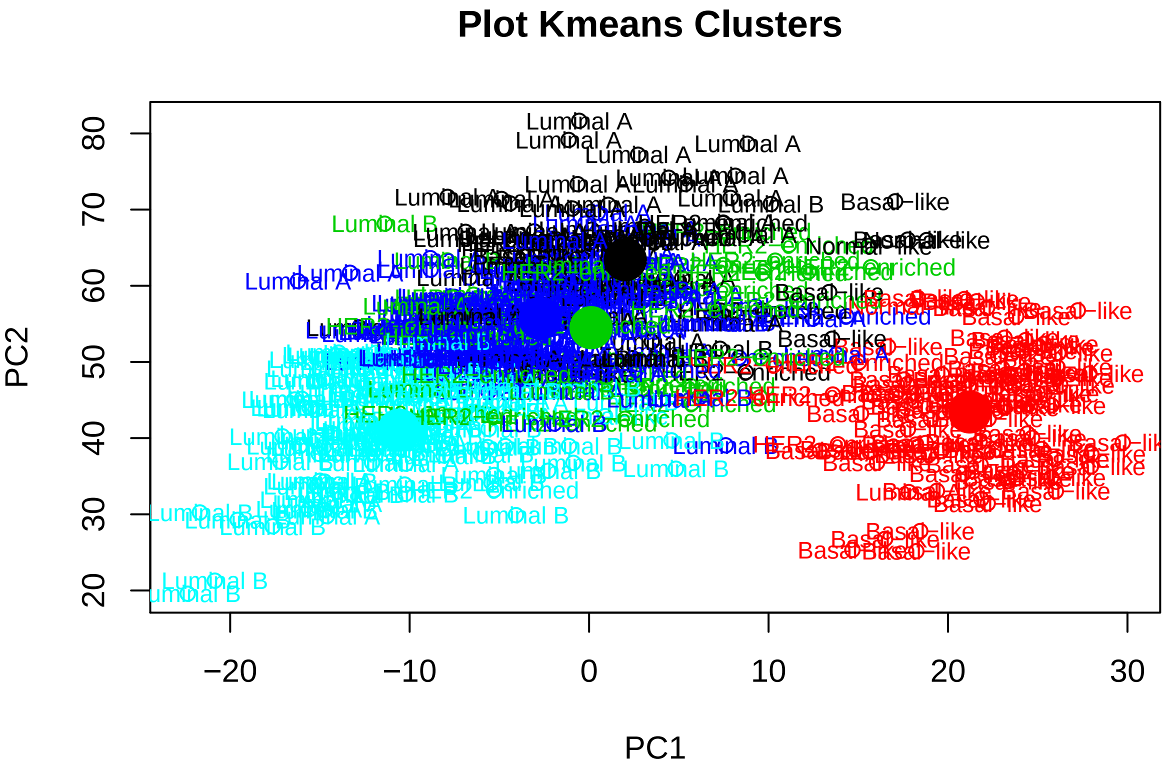
K-means

```
K = 5
km = kmeans(gdat,centers=K,nstart=25)
table(km$cluster,cdat$Subtype)
```

```
##
##     Basal-like HER2-enriched Luminal A Luminal B Normal-like
##   1          5             8        44        12           4
##   2         74             5         1         1           1
##   3          0            31         3         7           0
##   4          0             7       112        26           1
##   5          0             2        40        60           1
```

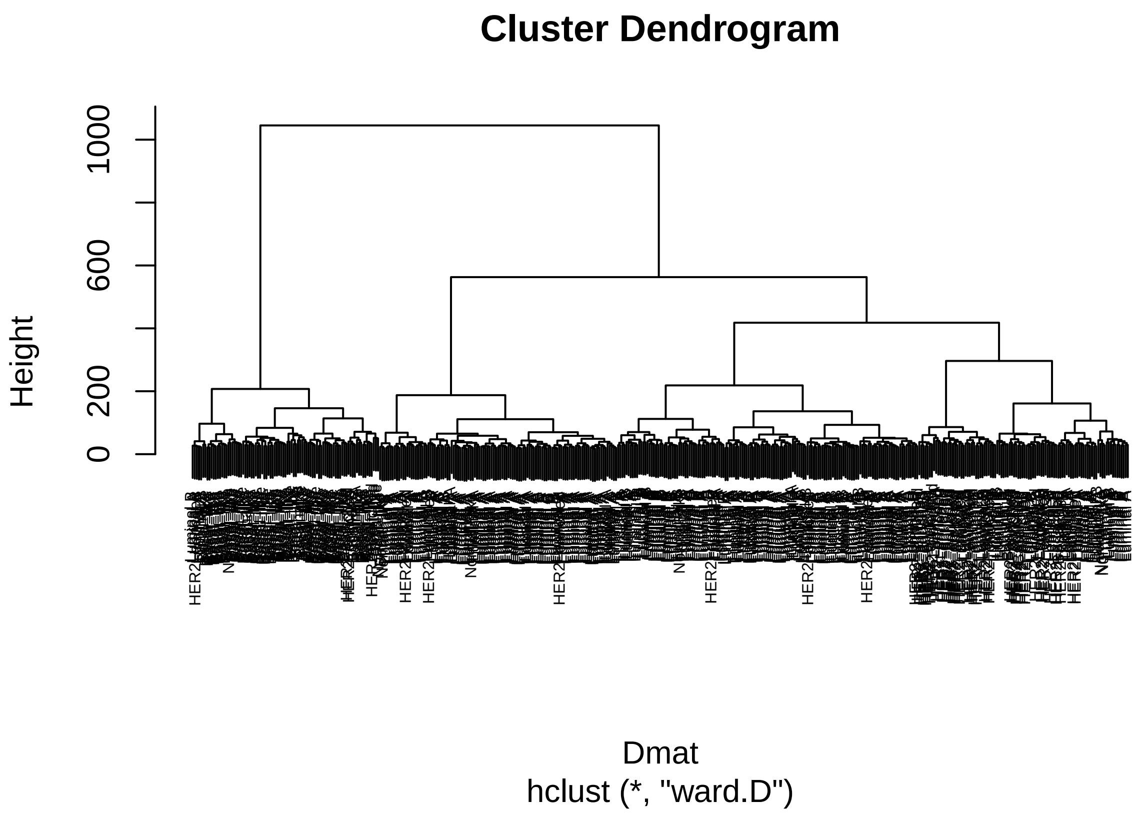
Plot Kmeans with labels

```
plot(Z[,1],Z[,2],col=km$cluster, main = "Plot Kmeans Clusters ", xlab = "PC1", ylab = "PC2")
text(Z[,1],Z[,2],cdat$Subtype,cex=.75,col=km$cluster)
cens = km$centers
points(cens%*%V[,1],cens%*%V[,2],col=1:K,pch=16,cex=3)
```



Hierarchical

```
#which linakge is the best?
#which distance metric is the best?

Dmat = dist(gdat)
com.hc = hclust(Dmat,method="ward.D")


plot(com.hc,labels=cdat$Subtype,cex=.5)
```



```
res.com = cutree(com.hc,5)
table(res.com,cdat$Subtype)
```

```
##
## res.com Basal-like HER2-enriched Luminal A Luminal B Normal-like
##       1          1             3        95        11           3
##       2          0             4        73        65           1
##       3         75             4         5         4           1
##       4          0            27         3         7           0
##       5          3            15        24        19           2
```

Consensus Clustering with Hierarchical

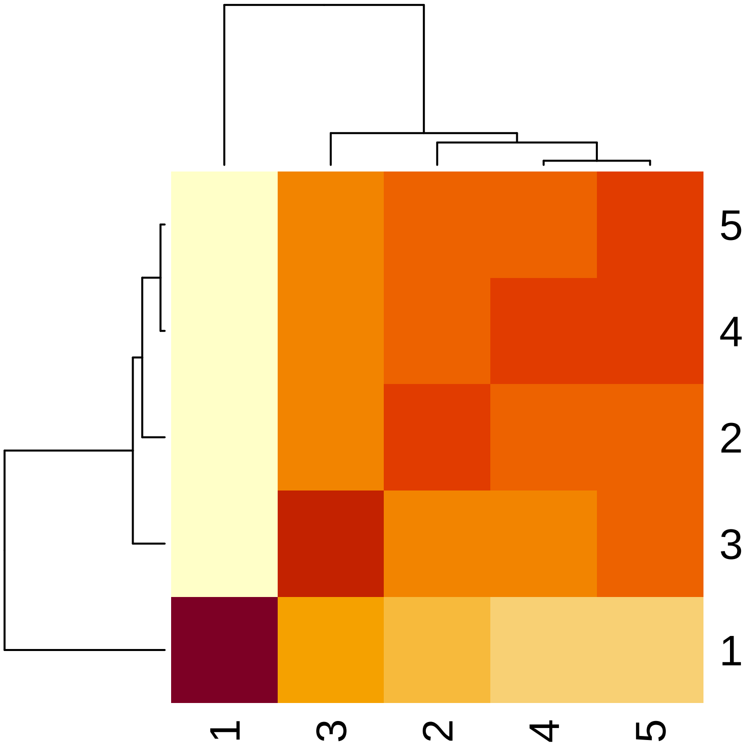
```
results = ConsensusClusterPlus(gdat,maxK=6,reps=500,pItem=0.8,pFeature=1,
clusterAlg="hc",distance="pearson",plot="png")
```

```
## end fraction
```

```
## clustered
## clustered
## clustered
## clustered
## clustered
```

Look at results for first 5 clusters

```
heatmap(results[[2]][["consensusMatrix"]][1:5,1:5])
```

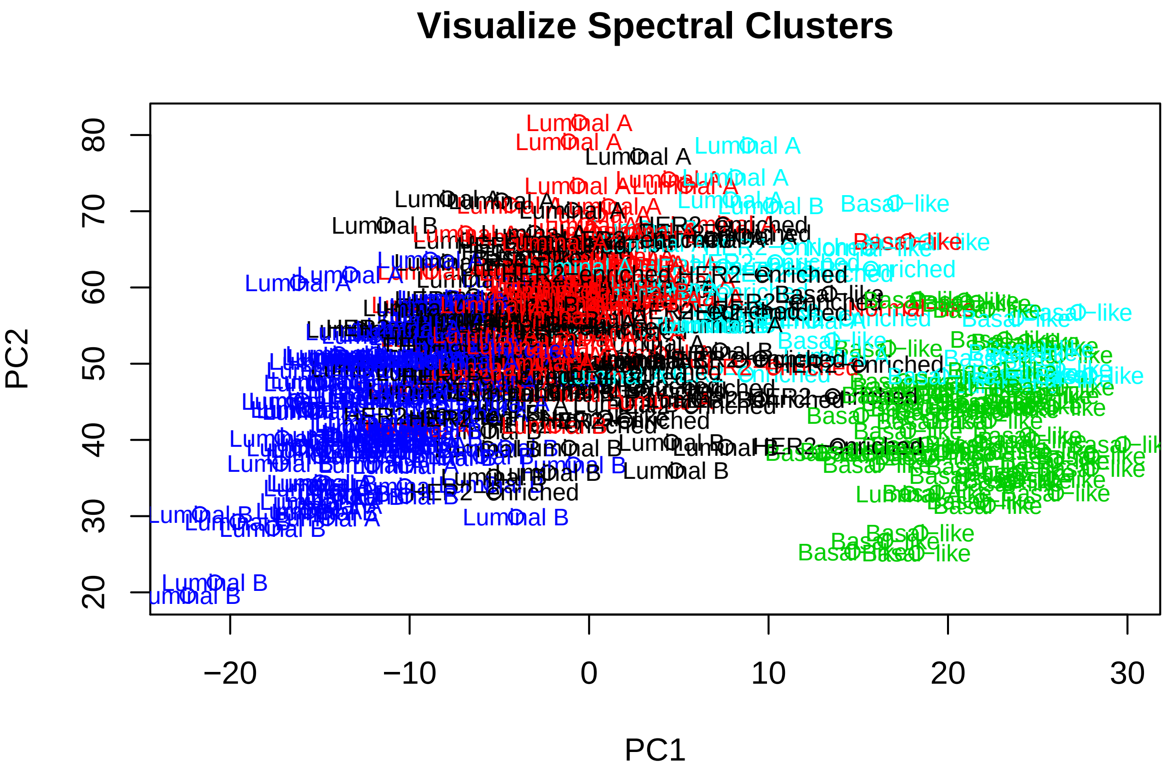


Spectral Clustering

```
K = 5
s_gdat = specClust(gdat, centers=K, nn = 7, method = "symmetric", gmax=NULL)
```

Visualize

```
plot(Z[,1],Z[,2],col=s_gdat$cluster, main = "Visualize Spectral Clusters", xlab = "PC1", ylab = "PC2")
text(Z[,1],Z[,2],cdat$Subtype,cex=.75,col=s_gdat$cluster)
```



Genes significantly associated with ER or PR Status, etc

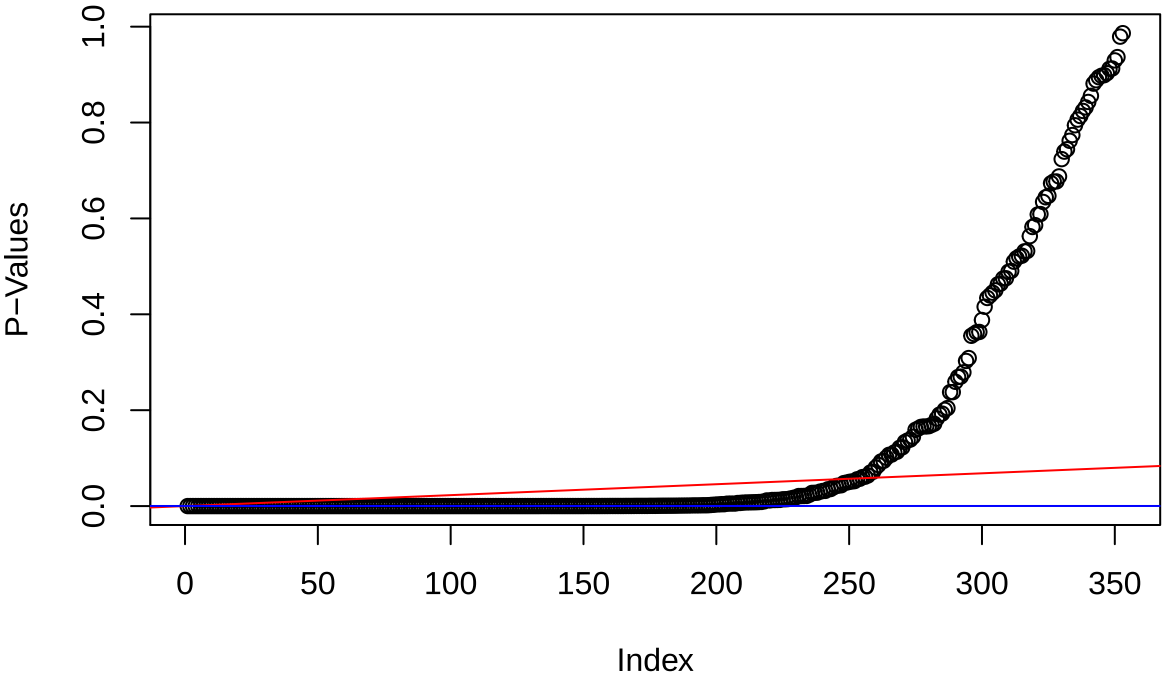
```r
x = gdat[cdat$ER=="Positive" | cdat$ER=="Negative",]
y.er = cdat$ER[cdat$ER=="Positive" | cdat$ER=="Negative"]
y.label = rep(1, length(y.er))
y.label[y.er == "Positive"]=2

ps = NULL
for(i in 1:ncol(gdat)) ps = c(ps,
 t.test(x[y.label==1,i],x[y.label==2,i])$p.value)
fdrs.bh = p.adjust(ps, method="BH")

cat("Number of Tests significant with alpha=0.1 using Bonferroni correction:",
sum(ps<0.1/length(y.label)), fill=TRUE)
```

```
## Number of Tests significant with alpha=0.1 using Bonferroni correction: 165
```

```r
cat("Number of Tests with FDR below 0.1:",
sum(fdrs.bh<0.1), fill=TRUE)
```

```
## Number of Tests with FDR below 0.1: 259
```

```r
plot(sort(ps,decreasing=FALSE),ylab="P-Values")
#BH procedure
abline(a=0, b=0.1/length(y.label),col="red")
#Bonferroni
abline(a=0.1/length(y.label), b=0,col="blue")
```



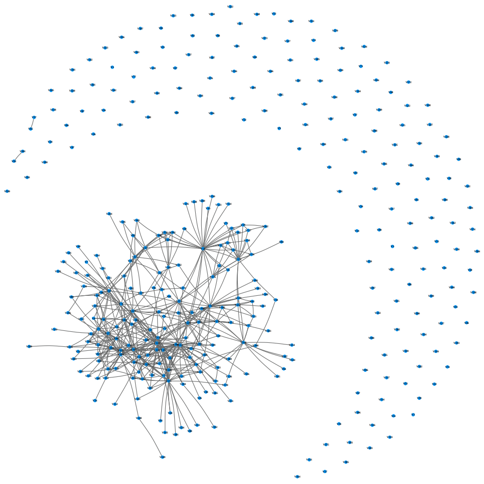
Graphical models - how are genes related?

```
#this takes a bit of time

lam = lambdaMax(gdat)*sqrt(log(ncol(gdat))/nrow(gdat))*0.01
net = XMRF(t(gdat),method="LPGM",lambda.path=lam,N=1,th=.0025)
```

```
## LPGM ::: Conducting sampling ... in progress:  100 %
## LPGM Completed.
```

```
plot(net)
```

```
## Plot optimal network
```



```
##                   [,1]        [,2]
##   [1,]  1.6355344883 27.85025999
##   [2,] 22.1442718477  4.14945599
##   [3,] 20.7325391667 15.62707111
##   [4,]  0.3598560423 10.72861394
##   [5,]  5.1391560551  6.80952548
##   [6,]  3.4422169535  7.05897462
##   [7,] -0.1282834254 14.56198277
##   [8,]  9.5211221830 14.02841568
##   [9,] 13.0120031442 23.67542195
##  [10,]  7.3526527684  7.57299682
##  [11,] -0.6999361967  8.46498822
##  [12,] 12.6524368851 31.49232595
##  [13,] 15.3526004893 29.68442138
##  [14,] 18.3112578970 16.69294316
##  [15,] 23.7452057943 15.96816252
##  [16,]  1.0395053223 24.91544632
##  [17,] 16.1281396299  0.27800071
##  [18,] 12.7739057304 11.98069403
##  [19,]  2.8573227816 14.64400438
##  [20,]  5.5804950852  4.27530664
##  [21,]  5.0686927237 29.21347417
##  [22,] 23.5885225347  6.23470186
##  [23,] 16.4248900544 21.03509625
##  [24,] -2.6084351886 22.75475378
##  [25,]  4.2226533269  9.94097810
##  [26,]  7.5784925085  5.28437187
##  [27,]  5.9206233502 27.79913018
##  [28,] 24.5952400448 20.27147894
##  [29,]  8.8551836752  9.53056592
##  [30,]  8.0147185082  5.70007630
##  [31,]  0.4531342332  5.11687151
##  [32,]  9.8772495984 15.38752244
##  [33,] 25.0270638272 11.50961393
##  [34,] 24.2595696269 14.16995406
##  [35,] 23.1327692716 20.23427271
##  [36,]  0.3443657267 23.29555877
##  [37,] 18.1738976934 24.60213176
##  [38,]  6.5466771640 16.83831716
##  [39,] 22.5617117435  5.27419366
##  [40,] 21.0985382263 19.89752113
##  [41,]  5.0942969586  8.56800782
##  [42,]  3.8874036855 15.53962816
##  [43,] 18.2052640547 20.63998893
##  [44,]  1.9397362829 10.14900066
##  [45,]  8.1633930409 18.22557646
##  [46,] 25.7895605083 10.42633628
##  [47,] -1.1159025759 22.37710522
##  [48,]  0.8827201102 12.50127593
##  [49,] -0.6924219515 15.61649468
##  [50,] 26.0177653066 14.01586359
##  [51,] 20.8060742491  4.07594192
##  [52,] 10.6121626727 24.26534039
##  [53,] 21.6173033651  6.27133936
##  [54,]  3.4594853156  8.10452981
##  [55,]  8.9302289180 14.31661088
##  [56,] 18.5726269366 18.34714539
##  [57,] -3.6985587235 24.42821428
##  [58,]  7.8126336438 15.47724606
##  [59,] 10.5453556205 17.09260802
##  [60,]  6.0121553724 24.75449840
##  [61,]  1.4094371593 12.60410787
##  [62,] 11.1737595869 11.87767645
##  [63,]  0.6451504885  8.28007292
##  [64,]  5.5007066431 12.23068504
##  [65,] -3.9320218053 23.63918190
##  [66,] -1.4918614707 23.88827957
##  [67,] 18.3682576202  5.63636809
##  [68,] 20.6183083388 26.13519340
##  [69,] 11.3425306906 28.54174756
##  [70,] 17.0874476532  3.53091091
##  [71,] 18.5791627064 27.91154127
##  [72,]  9.3769600816 17.20305914
##  [73,]  0.6887262754 10.00019828
##  [74,] 24.4816840752  7.41449948
##  [75,]  8.2979899652 11.56615772
##  [76,]  2.2411817578 11.71055191
##  [77,]  3.0935735090 27.18869478
##  [78,]  5.2052516208 10.02948225
##  [79,]  5.1472162610 10.85840509
##  [80,]  6.9181964665  3.04266924
##  [81,]  4.9557430721 30.51712792
##  [82,]  7.1104879112 29.99673042
##  [83,]  3.0428409212 16.36863824
##  [84,] 13.1639312085 15.93907290
##  [85,]  7.0828534808 31.39911672
##  [86,] 21.8865524120 13.04185493
##  [87,] -0.4194452525 24.86023051
##  [88,] -1.1552924225 12.18131486
##  [89,] 25.2473442252 21.57301658
##  [90,]  1.9077055506  9.33768160
##  [91,]  4.9122946732  3.73736625
##  [92,] -5.0715235450 21.44559765
##  [93,] 22.3666136531 18.81658058
##  [94,] 13.3261629948  7.27909549
##  [95,]  6.1400453715 11.09518916
##  [96,]  4.4600773327 12.80879221
##  [97,] 14.3833158677 22.28362374
##  [98,]  8.8212911931 30.00460976
##  [99,] 19.8328649857 16.75793563
## [100,]  7.7275969992 11.39202597
## [101,] 17.1367569264 19.37680545
## [102,]  4.6830622043 26.08403735
## [103,] 19.1837753835  1.88752236
## [104,]  2.2079863776  8.25217390
## [105,] 12.8763172165  6.77014233
## [106,] 11.3496739988 13.32198360
## [107,] 16.5328978270 24.32865643
## [108,] 11.2118417585 26.46872054
## [109,]  8.4705436427  8.89807478
## [110,] 14.7281207943 25.33764796
## [111,] 15.0624041923  1.07763955
## [112,]  1.8100519726  4.86738689
## [113,]  1.3795088679 13.48519428
## [114,] 19.9916391398  5.18164272
## [115,] 21.4128848908 23.90842725
## [116,]  6.6422499005 18.54721982
## [117,]  6.9245015835  8.06057554
## [118,] -2.3959057696 24.94802539
## [119,]  0.0001957319  6.80211319
## [120,]  1.8652565465  7.30766639
## [121,] 20.6728014180 14.06179531
## [122,] 21.7468496425 25.24031032
## [123,]  8.8733850655 18.48169428
## [124,]  4.6644937925  9.03443060
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```

```
## estimation using spacejam
net2 = SJ(gdat)
net2 = net2$G
sjnet <- 1*(net2)[,,1]
sjnet = graph.adjacency(sjnet, mode="undirected")
plot(sjnet, vertex.size=0.1, vertex.label=NA)
```